Endocrine Effects of PBDEs Investigated in Two Fish Species

R. V. Kuiper[1], A. D. Vethaak[2], R. F. Cantón[3], M. Dubbeldam[4], E-J van den Brandhof[5], P.E.G. Leonards[6], P. W. Wester[5] , M. van den Berg[3]
[1] Department of Pathobiology, Utrecht University, 3508 TD, The Netherlands
[2] National Institute for Coastal and Marine Management (RIKZ), 2500 EX The Hague, The Netherlands
[3] Institute for Risk Assessment Sciences (IRAS), Utrecht University, 3508 TD, The Netherlands
[4] Grontmij-AquaSense Laboratory for Aquatic Ecotoxicology, 4486 CX Colijnsplaat, The Netherlands
[5] National Institute of Public Health and the Environment (RIVM), 3720 BA Bilthoven, The Netherlands
[6] Institute for Environmental Studies (IVM), Vrije Universiteit Amsterdam, 1081 HV, The Netherlands

Introduction

Polybrominated dipenylethers (PBDEs) are widely used flame retardants. Although lower brominated technical products (PentaBDE mixtures) are no longer produced in Europa and the USA, earlier high production volumes have resulted in widespread contamination of the aquatic environment. Since bioavailability of PBDEs decreases with increasing bromine substitution, lower brominated diphenyl ethers are by far the most predominant PBDEs present in biota including fish (Birnbaum and Staskal, 2004).

In *in vitro* and terrestrial vertebrate test systems, exposure to PBDEs resulted in endocrine effects notably on the thyroid and sex steroid hormone systems (Birnbaum and Staskal, 2004; Hamers et al., 2006). With regard to the (eco)toxicological importance of these effects in aquatic vertebrates, the limited literature available indicates reduced spawning success and hepatic lipidosis in sticklebacks (*Gasterosteus acculeatus*) exposed to a commercial PBDE mix (Holm et al., 2003). A mechanistic background has not been provided for these observations. The present research addresses endocrine effects of a purified technical mix representing environmentally relevant PBDEs in two fish species *in vivo* under controlled laboratory conditions. European flounder (*Platichthys flesus*), a common species in European estuaries and coastal waters , were exposed for a relatively prolonged period (101 days) via food and sediment in an environmentally relevant test setup. Zebrafish (*Danio rerio*) were included as model fresh water species using a partial life-cycle setup that allows assessment of reproduction. All animals were subject to histological investigation with emphasis on thyroid, liver and reproductive organs. Plasma thyroid hormone concentrations were measured and enzymatic activity of cytochrome P450 aromatase (CYP19, estrogen synthetase) was measured in flounder gonads. Hepatic EROD activity was determined as an indicator of arylhydrocarbon receptor (AhR)-mediated toxicity. PBDE concentrations were chemically analyzed in all flounder and a representative selection of zebrafish to relate our observations to levels found in fish in the environment.

Materials and Methods

Commercial PentaBDE (DE-71, Great Lakes Chemical Corporation) was purified using activated charcoal, and absence of dioxin-like activity was confirmed using a dioxin-responsive reporter gene cell line (DR-CALUX).

Juvenile European flounder (10 months old, 48 ± 12g) were kept in sediment-containing aquariums under continuous flow-trough of estuarine water (3% saline). Exposure to PentaBDE was per group of 10 animals via spiked sediment and food according to a benchmark dosing protocol (Fig. 1). After euthanasia, length, weight and condition index were determined and plasma samples were collected

for analysis of thyroid hormones by radio immuno assay (RIA). Part of the liver and gonads were stored at -70°C before preparation of microsomes. EROD and aromatase activities were determined in liver and gonad microsomes respectively, as previously described (Fent et al., 1998; Heneweer et al., 2004). Remaining tissues including thyroid were routinely processed for histological examination. PBDE concentrations were chemically analyzed in muscle using GC/MS.
Adult reproducing zebrafish were exposed in 2*6 groups of 4 males and 4 females in all-glass aquariums containing 3L of fresh water. Exposure to PentaBDE was by adding stocks prepared in dimethylsulfoxide (DMSO) to the water shortly before semistatic renewal, twice weekly. Nominal concentrations were 0, 5, 16, 50, 160, and 500 µg/L. Immediately after renewal, sexes were placed together for 24 hrs and produced eggs were counted. Eggs gathered during the last week of parent exposure were allowed to hatch and juvenile survival and development were followed for 6 weeks at the same nominal exposure concentrations as their parents using a routine feeding protocol. After the exposure period the fish were euthanized and plasma was collected from adults after tail clipping. PBDE concentrations were chemically analyzed in homogenates of 1 adult fish of each sex and a pooled sample of 4 juveniles per concentration group. Remaining fish were routinely processed and histologically examined.

Results and Discussion

Internal PBDE concentrations increased linearly with exposure dose/concentrations as exemplified by BDE 47 concentrations in flounder muscle and zebrafish whole body homogenates and were not influenced by sex (Figs 1,2). The distribution of congeners was very constant in both species with BDE 47>BDE 99>BDEs 100, 49, 153, which is consistent with the general observations in the field (Boon et al., 2002). In zebrafish, a dose dependent increase of low levels of BDE 183 was noted, indicating that this congener can originate from PentaBDE mixtures. BDE 47 represented 82% of the ΣPBDE level in zebrafish, and 63% in flounder. Gross and microscopic morphological alterations related to exposure were not observed in either species, apart from curved spines in moribund zebrafish larvae exposed to high PBDE concentrations.

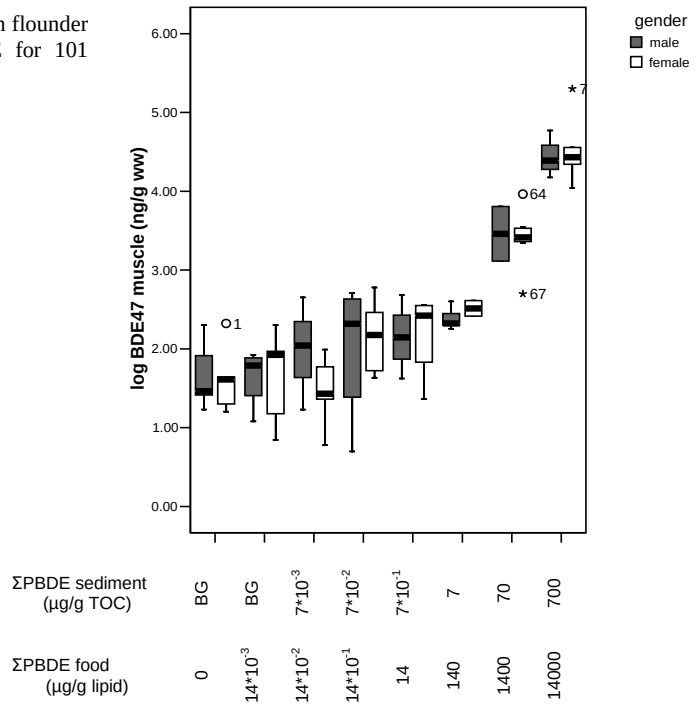
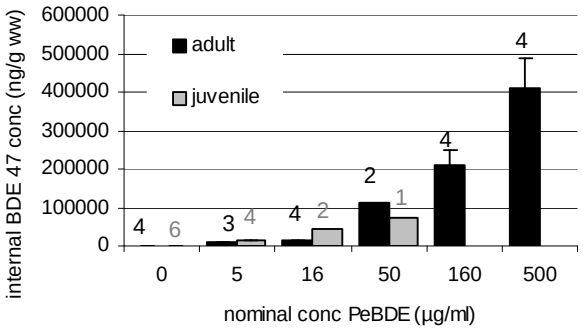


Figure 1. Internal BDE 47 concentrations in flounder (muscle) exposed to commercial PeBDE for 101 days. BG=background.

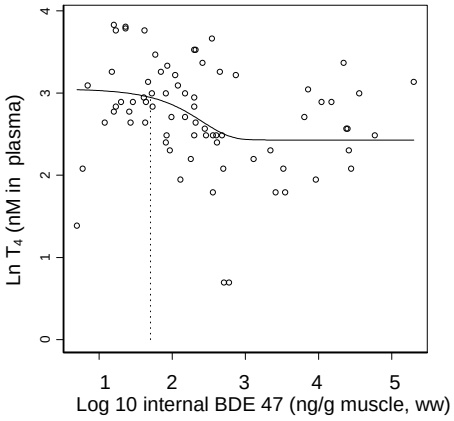
Figure 2. Internal BDE 47 concentrations in zebrafish (whole animal homogenates) exposed to commercial PeBDE for 30 days (adults) and 45 days (juveniles). Error bars represent standard deviations. Numbers above bars are number of replicates.



In flounder plasma, a significant negative dose response in plasma thyroid hormone T_4 levels was indicated (Fig. 3). A 10% T_4 reduction was calculated as early as at an internal dose of 51 ng BDE 47/g muscle (wet weight, ww). The maximum effect size was limited to 46% reduced T_4 concentrations. T_3 concentrations were not affected.

Figure 3. Relation between T_4 and internal BDE 47 concentrations in flounder (muscle). The stippled line indicates the BDE 47 concentration at which the T_4 concentration is reduced by 10%.

Model 4 according to Slob (2002):
$y = a[c-(c-1)e^{-bx}]$ with
a=21.16
b=4.8
c=0.54
log likelihood=-65.83



Aromatase activities in flounder ovaries likewise showed a mild decrease with increasing internal BDE 47 concentrations (Fig. 4). The significant dose response model calculated a 10% decrease already at an internal BDE 47 concentration of 40 ng/g muscle (ww), and a maximum reduction of 46%. No effect was observed on aromatase activity in testes. EROD activity was not induced consistent with observations in mammalian systems (van den Berg et al., 2006).
In contrast, a dose dependent increase of both thyroid hormones T_3 and T_4 was found in zebrafish exposed to PBDEs. High T_4 concentrations in particular were more obvious at internal BDE 47 concentrations around and exceeding the µg/g ww level. Although not statistically significant, egg production appeared to decrease with increasing PBDE exposure concentration (Fig. 5). In addition, survival of zebrafish larvae exposed to 160 and 500 µg PBDE/L was significantly reduced. At 50 µg/L (internal concentration: 73 µg/g ww) no reduced survival was noted. The highest reported PBDE levels in fish were in the low µg/g range in fresh water burbot (*Lota lota;*Mariussen et al., 2003). Our present results indicate that the thyroid system and successful production of offspring in freshwater zebrafish may be affected by PBDEs, at only slightly higher internal concentrations. Although effects in flounder may occur at lower internal concentrations, PBDE levels in marine fish are usually in the low ng/g range (*e.g.* Boon et al., 2002). The limited effect sizes at these internal concentrations and the absence of accompanying behavioral and histopathological changes may indicate a limited risk for both fresh and salt water species at current environmental PBDE exposure levels.

Figure 4. Relation between aromatase activity in ovaries and internal BDE 47 concentrations in muscle of female flounder. The vertical stippled line indicates the internal BDE 47 concentration at which a 10% decrease of aromatase activity was calculated.

Model 4 according to Slob (2002):
$y = a[c-(c-1)e^{-bx}]$ with
a=1.73
b=5.13
c=0.46
log likelihood=-41.4

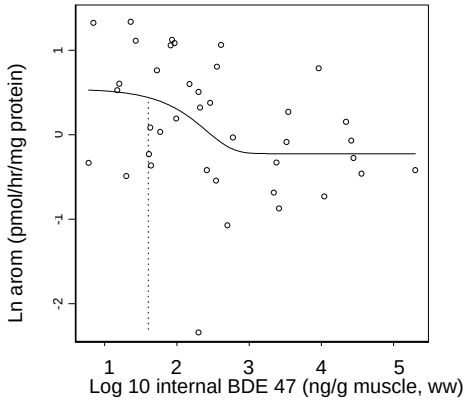
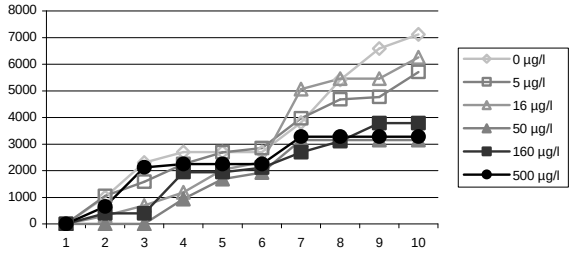


Figure 5. Total cumulative egg production in zebrafish exposed to DE-71 for 30 days.



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